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Discovery of Novel Cellodextrin Phosphorylases – Where Bioinformatics Meets Biochemistry

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Millions of protein sequences are being discovered at an incredible pace, providing a wide pool of potential biocatalysts that can be used in the industry. Here, we explored the sequence space of Glycoside Hydrolase Family 94 (GH_94) with the aim of discovering novel cellodextrin phosphorylases, enzymes that can be applied for biocatalytic production of cello-oligosaccharides, indigestible carbohydrates with potential applications in various industries. For this purpose, all full-length protein sequences classified in the family GH_94 and originating from bacteria and archaea were bioinformatically extracted from the CAZy database (http://www.cazy.org/) and used to create a protein phylogenetic tree. Specific amino acid residues that can pinpoint towards catalytic preference of an enzyme, known as “specificity fingerprints”, were used as a guide to distinguish between approximately 1000 unique protein sequences allocated in the GH_94. Eighteen putative cellodextrin phosphorylases were recombinantly expressed in *E. coli*, of which eight enzymes were found to express well and were afterwards characterized in detail. This work not only expanded a modest collection of previously described cellodextrin phosphorylases but also demonstrated a proof of concept with respect to modern enzyme discovery strategies.

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